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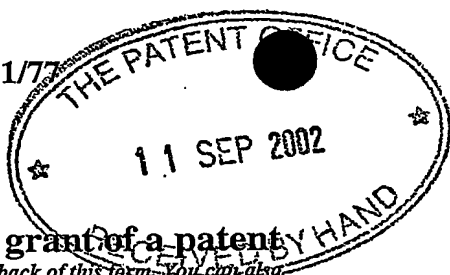
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020-7242 8692

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COMPLEXES OF ANIONIC POLYSACCHARIDES WITH SILVER

The present invention relates to complexes formed between anionic polysaccharides, such as oxidized regenerated cellulose (ORC), and silver, and to
5 the uses thereof for the treatment of wounds.

Anionic polysaccharides such as alginates, hyaluronic acid and its salts, and oxidized celluloses such as oxidized regenerated cellulose (ORC) are known for use as wound dressings. Alginates and ORC are hemostatic when applied to a
10 wound.

EP-A-0437095 describes the preparation of a neutralized ORC fabric by reacting ORC with a buffered solution of a salt of a weak acid, such as sodium acetate. The use of a buffered reagent is said to result in less breakdown of ORC fabrics.
15 The neutralized ORC can support further acid-sensitive hemostatic agents such as thrombin or t-PA.

WO98/00180 describes the use of freeze-dried sponges of collagen admixed with oxidized regenerated cellulose (ORC) for the treatment of chronic wounds. The
20 mixed sponges provide enhanced healing of chronic wounds such as venous ulcers, decubitis ulcers and diabetic ulcers. There is also a collagen/alginate wound dressing that has been commercially available for some years under the registered trade mark KALTOSTAT.

25 The present invention provides a complex of an anionic polysaccharide with silver.

The term "complex" refers to an intimate mixture at the molecular scale, preferably with ionic or covalent bonding between the silver and the polysaccharide. The complex preferably comprises a salt formed between the anionic polysaccharide
30 and Ag^+ , but it may also comprise silver clusters and/or colloidal silver metal, for example produced by exposure of the complex to light.

Preferably, the anionic polysaccharide is a polycarboxylate. Suitable anionic polysaccharides include alginates, hyaluronates, pectins, carrageenans, xanthan gums, sulfated dextrans, cellulose derivatives such as carboxymethyl celluloses, and oxidized celluloses.

5

The term "oxidized cellulose" refers to any material produced by the oxidation of cellulose, for example with dinitrogen tetroxide. Such oxidation converts primary alcohol groups on the saccharide residues to carboxylic acid groups, forming uronic acid residues within the cellulose chain. The oxidation generally does not proceed with complete selectivity, and as a result hydroxyl groups on carbons 2 and 3 are occasionally converted to the keto form. These keto units introduce an alkali-labile link, which at pH 7 or higher initiates the decomposition of the polymer via formation of a lactone and sugar ring cleavage. As a result, oxidized cellulose is biodegradable and bioabsorbable under physiological conditions.

15

The preferred oxidized cellulose for use in the present invention is oxidized regenerated cellulose (ORC) prepared by oxidation of a regenerated cellulose, such as rayon. It has been known for some time that ORC has haemostatic properties. ORC has been available as a haemostatic fabric called SURGICEL (Registered Trade Mark of Johnson & Johnson Medical, Inc.) since 1950. This product is produced by the oxidation of a knitted rayon material.

Preferably, the anionic polysaccharide is substantially insoluble in water at pH7. Preferably, the anionic polysaccharide has a molecular weight greater than about 20,000, more preferably greater than about 50,000. Preferably, the anionic polysaccharide is in the form of a film, or fibers having length greater than 1mm.

Preferably the amount of silver in the complex is from about 0.01 to about 50% by weight based on the weight of the anionic polysaccharide; more preferably from about 1% to about 40%, still more preferably from about 2% to about 30% by weight, and most preferably from about 5% to about 25%.

It has been found that the complexes according to the present invention are suitable for use as antimicrobial wound dressings. The silver confers antimicrobial properties on the complex. More surprisingly, at low concentrations the silver in the complex exhibits a proliferative effect on wound healing cells and therefore is expected to promote wound healing even when the complexes according to the invention are applied directly to a wound. The oligodynamic effect of silver on cells in culture is known – this is usually regarded as affecting the growth of both bacteria and cells. It is therefore surprising to find that silver in the dressings of the present invention can have the opposite effect on certain cells of importance to wound healing.

Accordingly, in a second aspect, the present invention provides a wound dressing comprising a complex of an anionic polysaccharide with silver. Preferably, the complex is one of the preferred complexes as hereinbefore described in connection with the first aspect of the invention.

Preferably, the wound dressing is in the form of a sheet, for example a sheet of substantially uniform thickness. The area of the sheet is typically from about 1 cm² to about 400cm², and the thickness typically from about 1mm to about 10mm. The sheet may for example be a freeze-dried sponge, or a knitted, woven or nonwoven fibrous sheet or a gel sheet. Preferably, the sheet comprises less than about 15% by weight of water.

Preferably, the wound dressing comprises from about 0.1wt% to 100wt% of the complex, more preferably from about 0.1wt% to about 5wt.%. Preferably, the amount of silver in the wound dressing is from about 0.01wt% to about 3wt.%, more preferably from about 0.1wt% to about 0.6wt.%, and most preferably about 0.3wt.%. Lesser amounts of silver could give insufficient antimicrobial effect. Greater amounts of silver could give rise to antiproliferative effects on wound healing cells.

The dressings according to the present invention may further comprise one or more polysaccharides that have not been complexed to silver. These

polysaccharides may include any one or more of the anionic polysaccharides listed above as being suitable for complexation with silver. The additional polysaccharides may additionally or alternatively comprise any other medically acceptable polysaccharides, such as for example cellulose, regenerated celluloses
5 such as rayon, non-anionic cellulose derivatives such as hydroxyethyl cellulose, and starch derivatives.

The dressings according to the present invention may further comprise anionic polysaccharides that have been complexed to therapeutically effective metal ions
10 other than silver, for example bismuth, copper, nickel, zinc, manganese, magnesium, gold, or mixtures thereof. Preferably, the amounts of such polysaccharides complexed to other metals is from 0.001 to 10wt.% of the dressing, more preferably from 0.01 to 1wt% of the dressing. Preferably, the amounts of said other metals is from 10 to 10000ppm, more preferably from about
15 50 to about 1000ppm in the dressing.

The dressings according to the present invention may further comprise other medically acceptable materials besides polysaccharides, including textile fibers such as nylon or polyester staple fibers, nontextile fibers such as meltblown nylon
20 fibers, and bioresorbable fibers such as polylactide/polyglycolide fibers. The other fibers may provide reinforcement and dilution of the polysaccharide. The other fibers are preferably present in an amount up to about 90wt%, more preferably from about 25% to about 75wt% of the dressing.

25 The dressings according to the present invention preferably further comprise collagen, in addition to the silver complex of an anionic polysaccharide. Preferably the amount of collagen in the dressing is from about 10% to about 90% by weight based on the dry weight of the dressing, more preferably from about 25% to about 75%.

30

The collagen may be selected from native collagens such as Types I, II or III native collagens, atelopeptide collagen, regenerated collagen and gelatin.

In certain embodiments, at least a portion of the collagen in the dressing is also complexed with silver. This can be achieved by treating the collagen with a solution of a silver salt. The silver salt may for example be silver acetate or silver nitrate at a concentration of about 0.01 molar to about 1 molar. The treatment is preferably carried out at a pH of from about 5 to about 9. It is thought that the silver complexes primarily to the nitrogen-containing side chains of the collagen amino acids, in particular to lysine, hydroxylysine, asparagine, glutamine and arginine. The silver could also bind to the sulfhydryl groups of methionine and cysteine residues, where present, and to carboxyl groups of aspartate and glutamate.

Preferably the amount of silver in the collagen complex is from about 0.01 to about 30% by weight based on the weight of the collagen, more preferably from about 0.1% to about 20%, more preferably from about 2% to about 10% by weight. Preferably, the amount of silver-collagen complex in the wound dressing is from about 0.1 to about 10 wt.%, more preferably from about 0.1 to about 2wt.%.

Preferred wound dressings according to the present invention are silver-containing versions of the dressings according to WO98/00180 or EP-A-1153622, the entire contents of which are incorporated herein by reference. Briefly, these dressings are freeze-dried sponges that comprise a mixture of collagen and ORC, and that preferably consist essentially of such a mixture. Preferably, the weight ratio of collagen to ORC is about 40:60 to about 60:40. In the sponges according to the present invention, a portion of the ORC is replaced by a silver/ORC complex, and/or a portion of the collagen may be replaced by a silver/collagen complex. Preferably, from about 0.1 to about 50wt.% of the ORC may be replaced by the silver-ORC complex, and a similar fraction of the collagen may similarly be replaced. It will be appreciated that silver ion exchange can take place between the collagen and the ORC in such collagen/ORC wound dressings.

30

In certain embodiments the dressing according to the present invention is fully resorbable. Preferably, the wound dressings according to the present invention are suitable for application directly to a wound surface.

Preferably, the wound dressings according to the present invention are sterile. Preferably, they are packaged in a microorganism-impermeable container. Preferably, the sterility assurance level is better than 10^{-6} . Preferably, the
5 dressing has been sterilized by gamma-irradiation.

In a further aspect, the present invention provides the use of a complex of an anionic polysaccharide with silver for the preparation of a dressing for the treatment of wounds, especially chronic wounds such as venous ulcers, decubitis
10 ulcers or diabetic ulcers. Preferably, the medicament is a wound dressing according to the present invention. Preferably, the treatment comprises applying the dressing directly to the surface of the wound.

In a further aspect, the present invention provides a method of treatment of a
15 wound, comprising applying to the wound a wound dressing comprising an effective amount of a complex of an anionic polysaccharide and silver, whereby said complex is antimicrobially effective without exhibiting substantial antiproliferative activity against wound healing cells. The method is especially suitable for treatment of chronic wounds such as venous ulcers, decubitis ulcers
20 or diabetic ulcers.

In a further aspect, the present invention provides a method of making a complex of an anionic polysaccharide and silver, comprising the step of treating an anionic polysaccharide with a solution of a silver salt. Preferably, the solution is an
25 aqueous solution.

Preferably, the anionic polysaccharide is substantially insoluble in water at pH7, and the treatment is therefore carried out on the polysaccharide in the solid state. For example, the polysaccharide may be in the form of solid fibers, sheet, sponge
30 or fabric. In certain embodiments, the anionic polysaccharide is a salt and the treatment therefore can be regarded as an ion exchange. In other embodiments, the anionic polysaccharide is at least partly in free acid form, in which case the silver salt is preferably a salt of a weak acid, for example silver acetate, whereby

the anionic polysaccharide is at least partially neutralised by the silver salt. Similar processes are described in EP-A-0437095, the entire content of which is expressly incorporated herein by reference.

- 5 The neutralization reaction can be carried out in water or alcohol alone but is preferably carried out in mixtures of water and alcohols. The use of a mixture of water and alcohol provides good solubility for the weak acid salts via the water, and the alcohol prevents the anionic polysaccharide from excessively swelling, distorting and weakening during the neutralization. Thus the physical properties of
- 10 the material are retained. Methanol is the preferred alcohol because many of the above-mentioned salts have good solubility in this alcohol in combination with water. Preferably, the alcohol to water ratio has a range of about 4:1 to 1:4 . If the solution becomes too rich in alcohol, some salts may no longer be soluble particularly if the alcohol is other than methanol. If the solution becomes too rich in
- 15 water, some swelling of the polysaccharide will occur as neutralization takes place and there will be some loss in physical properties such as in the tensile strength of the polysaccharide. Other useful alcohols include, for example, ethyl alcohol, propyl alcohol and isopropyl alcohol.
- 20 The use of a mild neutralizing agent such as silver acetate allows for control of the degree of neutralization. Use of stoichiometric and chemically equivalent amounts of neutralizing agent and carboxylic acid on the anionic polysaccharide does not produce a 100% neutralized polysaccharide as would be produced with strong irreversible reactions with bases such as sodium hydroxide, sodium carbonate,
- 25 sodium bicarbonate and ammonium hydroxide.

Anionic polysaccharides behave as an ion exchanger and will pull out of solution the silver cation of any silver salt that is passed over them. The by-product of this exchange is an acid from the salt and by using a salt of a weak organic acid, a

- 30 weak acid such as acetic acid is produced which does no damage to the polysaccharide. Using salts of strong acids such as sodium chloride or sodium sulfate produces hydrochloric acid or sulfuric acid by-products respectively, and

these strong acids can cause damage such as depolymerization of the polysaccharide

When using silver salts of weak acids, the silver ion is exchanged for a proton on the polysaccharide and part of the salt is converted to weak acid. The mixture of acid and salt in the solution results in a buffered solution which maintains a fairly constant pH and controls the degree of neutralization. An equilibrium reaction is established whereby the silver ions are bound to the acid portion of the polysaccharide and also to the salt molecules. This partitioning of the silver ions prevents the neutralization of the polysaccharide from going to completion.

Using a stoichiometric amount of, for example, silver acetate brings about a 65-75% degree of neutralization of the carboxylic acid groups on an oxidized cellulose polymer. This control of pH by creating a self generating buffered solution and the use of methanol to control the swelling of the material, leads to a partially neutralized material in which the physical properties, e.g. tensile strength and shape of the polysaccharide, are preserved.

The amount of silver salt used is generally about equal to or up to twice the stoichiometric amount of carboxylic acid content of the polysaccharide. Alternatively, a second charge of a stoichiometric amount of silver salt can be used if the reaction is recharged with fresh solvent and salt after the first charge reaches a constant pH. The material with elevated pH is then washed to remove the excess silver salt and ions therefrom.

25

It will be appreciated that the complexes obtainable by the processes of the present invention may be used in the products and methods according to any other aspect of the invention. More generally, any feature or combination of features that is described as preferred in connection with any one aspect of the invention is also preferred in connection with every other aspect of the invention. Furthermore, any combination of the specific or preferred features disclosed herein is also encompassed within the scope of the disclosure.

A specific embodiment of the process and product according to the present invention will now be described further, by way of example, with reference to the accompanying drawings, in which:

Figure 1 shows antimicrobial effect against *Staphylococcus Aureus*, plotted as
5 zone of inhibition in mm at 2 days, for a first range of wound dressing materials;

Figure 2 shows antimicrobial effect against *Staphylococcus Aureus*, plotted as zone of inhibition in mm at 24 and 48 hours, for a second range of wound dressing materials;

Figure 3 shows antimicrobial effect against *Pseudomonas Aeruginosa*, plotted as
10 zone of inhibition in mm at 24 and 48 hours, for the second range of wound dressing materials;

Figure 4 shows the effect on cell proliferation of extracts from a third range of wound dressing materials;

Figure 5 shows antimicrobial effect against *Staphylococcus Aureus*, plotted as
15 zone of inhibition in mm at 2 days, for a fourth range of wound dressing materials; and

Figure 6 shows the effect on cell proliferation of extracts from a fifth range of wound dressing materials.

20 Example 1

A complex of ORC with silver was prepared as follows.

A SURGICEL cloth (Johnson & Johnson Medical, Arlington) was milled using a
25 rotary knife cutter through a screen-plate, maintaining the temperature below 60°C, to provide a fibrous ORC powder

Silver acetate powder (4.08g) was dissolved in 800mls of de-ionised water. After all the powder had dissolved to form a colourless, clear solution, the ORC milled
30 fibers (5g) were added to this solution. The ORC was then allowed to react for sixty minutes, or less if desired. The solution was then filtered and the fibers were collected and washed with either de-ionised water or alcohol. The silver complex of ORC was then allowed to dry at 37°C overnight or until the fibers were dry. To

reduce the darkening of the final product the solution and reacted fibers were protected from the light throughout the reaction.

Examples 2-7

5

Freeze-dried collagen/ORC sponges suitable for use as wound dressings were prepared as follows.

First, the collagen component was prepared from bovine corium as follows.

10 Bovine corium was split from cow hide, scraped and soaked in sodium hypochlorite solution (0.03% w/v) to inhibit microbial activity pending further processing.

The corium was then washed with water and treated with a solution containing
15 sodium hydroxide (0.2% w/v) and hydrogen peroxide (0.02% w/v) to swell and sterilize the corium at ambient temperature.

The corium splits then underwent an alkali treatment step in a solution containing sodium hydroxide, calcium hydroxide and sodium bicarbonate (0.4% w/v,
20 0.6% w/v and 0.05% w.v, respectively) at pH greater than 12.2, ambient temperature, and for a time of 10-14 days, with tumbling, until an amide nitrogen level less than 0.24mmol/g was reached.

The corium splits then underwent an acid treatment step with 1% hydrochloric acid
25 at ambient temperature and pH 0.8-1.2. The treatment was continued with tumbling until the corium splits had absorbed sufficient acid to reach a pH less than 2.5. The splits were then washed with water until the pH value of corium splits reached 3.0-3.4.

30 The corium splits were then comminuted with ice in a bowl chopper first with a coarse comminution and then with a fine comminution setting. The resulting paste, which was made up in a ratio of 650g of the corium splits to 100g of water, as ice, was frozen and stored before use in the next stage of the process.

However, the collagen was not freeze-dried before admixture with the ORC in the next stage.

The silver-ORC complex, unmodified milled ORC powder, and the required weight
5 (according to solids content) of frozen collagen paste were then added to a sufficient amount of water acidified with acetic acid to obtain a pH value of 3.0 and a total solids content of 1.0%-2.0%, in the following proportions by weight:

Example 2: 45% Silver-ORC / 55% Collagen

10 Example 3: 20% Silver-ORC + 15% ORC / 55% Collagen

Example 4: 10% Silver-ORC + 35% ORC / 55% Collagen

Example 5: 2% Silver-ORC + 43% ORC / 55% Collagen

Example 6: 1% Silver-ORC + 44% ORC / 55% Collagen

Example 7: 0.2% Silver-ORC + 44.8% ORC / 55% Collagen

15

The mixture was homogenized through a Fryma MZ130D homogenizer, progressively diminishing the settings to form a homogeneous slurry. The pH of the slurry was maintained at 2.9-3.1. The slurry temperature was maintained below 20°C, and the solids content was maintained at $1\% \pm 0.07$.

20

The resulting slurry was pumped to a degassing vessel. Vacuum was initiated for a minimum of 30 minutes, with intermittent stirring, to degas the slurry. The slurry was then pumped into freeze-drier trays to a depth of 25mm. The trays were placed onto freezer shelves where the temperature has been preset to -40°C. The
25 freeze-drier programme was then initiated to dry and dehydrothermally cross-link the collagen and ORC to form thick sponge pads.

On completion of the cycle, the vacuum was released, the freeze-dried blocks were removed, and were then split to remove the top and bottom surface layers,
30 and to divide the remainder of the blocks into 3mm-thick pads. The step of splitting the freeze-dried blocks into pads was carried out with a Fecken Kirfel K1 slitter.

Finally, the pads were die-cut to the desired size and shape on a die-cutter, packaged, and sterilized with 18-29 KGy of cobalt 60 gamma-irradiation. Surprisingly, this irradiation does not cause significant denaturation of the collagen, which appears to be stabilized by the presence of ORC. The resulting
5 freeze-dried collagen ORC pads had a uniform, white, velvety appearance.

Examples 8-11

Collagen/ORC sponges were prepared from unmodified ORC and from collagen,
10 wherein a fraction of the collagen had been replaced by a collagen-silver complex, as follows.

Silver acetate powder (1.48g) was dissolved in 400mls de-ionised water with stirring and warming.

15

Collagen slurry (417g, containing 5g solids at the percentage solids in the slurry of 1.2%) was added to the silver acetate solution and allowed to react for ten minutes stirring gently (If the collagen was allowed to react for longer it become stringy and was difficult to re-slurry in acetic acid).

20

The solution was filtered using the funnel and filter paper and the solids rinsed with de-ionised water. Squeezed out excess liquid in the solid by gently pressing between two filter papers. The collagen solids were protected from the light during this step and all following steps.

25

The solids were made up to a weight of 417g using 0.05M acetic acid. The mixture was placed into an Industrial Waring Blender and blended until a smooth slurry was achieved again.

30 Freeze dried sponges were then made as described above for Examples 2-7, with unmodified ORC and with replacement of a fraction of the collagen by the silver-complexed collagen slurry. These examples were considered to fall within the

scope of the present invention because the silver in the silver-collagen complex will also complex and exchange with the ORC under the preparation conditions.

The following formulations were made:

5

Example 8: 55% Silver-Collagen / 45% ORC

Example 9: 45% Silver-Collagen + 10% Collagen / 45% ORC

Example 10: 30% Silver-Collagen + 25% Collagen / 45% ORC

Example 11: 15% Silver-Collagen + 40% Collagen / 45% ORC

10

Procedure 1

The bactericidal activity of the sponges prepared in Examples 2 to 7 was tested on *pseudomonas Aeruginosa* and *staphylococcus Aureus* by looking at zone of inhibition.

15

Six 2cm x 2cm squares of each sample were cut out in sterile conditions. On day one of the experiment cultures of both *Pseudomonas aeruginosa* and *Staphylococcus aureus* were incubated aerobically at 37°C for 24 hours on Diagnostic Sensitivity Agar (DSA). After 24 hours test samples were each placed on a DSA plate and were immediately wetted with 0.5mls of a buffer solution. Three squares of sample were placed on plates inoculated with *Pseudomonas aeruginosa* and three were placed on plates inoculated with *Staphylococcus aureus*. The plates were then incubated at 37°C for 24 hours. The zone of inhibited growth around the sample was then measured using callipers and the test sample was placed on a new inoculated DSA plate. A swab test was carried out on the area beneath the sample to determine if the sample was bacteriostatic if not bactericidal by smearing the swab on a DSA plate and incubating it for 24 hours and then examining the growth. The samples were transferred onto fresh inoculated plates with the above procedure being carried out every 24 hours for 72 hours as long as the samples remain intact.

20

25

30

As a negative control, a freeze dried sponge of 45%ORC/55%collagen without any silver was tested. A commercially available silver-containing antimicrobial dressing (ACTICOAT, registered trade mark of Smith & Nephew) and silver nitrate solution (0.5%) were used as positive controls and zones of inhibition were
5 observed for both over the test period.

Referring to Figures 1 and 2, it can be seen that significant bactericidal effects were observed against *Staphylococcus aureus* for the materials containing 1% silver-ORC and above. The performance was comparable to that of the
10 ACTICOAT dressing.

Referring to Figure 3, it can be seen that significant bactericidal effects were observed against *Pseudomonas Aeruginosa* for the materials containing 10% silver-ORC and above. The performance at 24 hours was superior to that of the
15 ACTICOAT dressing.

The bactericidal activity of the sponges prepared in Examples 8, 10 and 11 was tested on *Pseudomonas Aeruginosa* by looking at zone of inhibition in the same way as described above. Again, a 55%collagen/45%ORC sponge was used as a
20 negative control, and silver nitrate solution (0.5%) and ACTICOAT were used as positive controls. The results at 48 hours were shown in Figure 5. It can be seen that the sponges made with collagen-silver complexes show similar zones of inhibition to the positive controls and to the silver-ORC examples of Fig. 3.

25 Procedure 2

The antiproliferative effects of the dressings from the above Examples were assessed as follows.

30 Prototype extracts were prepared as follows - 1mg of each wound dressing to be tested was placed in 1ml of serum free medium and incubated for 24 hours at 37°C under sterile conditions.

Adult human dermal fibroblasts were grown and maintained in DMEM 10% FBS (standard culture medium; Dulbecco's minimal essential medium; foetal bovine serum). These cells were routinely subcultured and used for experimental testing when 95% confluent. Adult human dermal fibroblasts were harvested at 95%
5 confluency and re-seeded in a 96-well microtitre plate (100µl/well) in DMEM + 10% FBS at a cell density of 2.5×10^4 cells/ml. Cells were allowed to adhere to the plate surface for 24 hours in a humidified incubator at 37°C, 5% CO₂. The medium was then removed by aspiration and the cell monolayer washed with serum-free DMEM. Test samples (extracts of each prototype) were then added to the cell
10 monolayer (100µl/well), and 6 replicates of each concentration tested. Serum-containing growth medium (10% FBS in DMEM) was used as a positive control, and serum-free medium was used as a negative control. All samples were incubated with the cells for 48 hours at 37°C, 5% CO₂. After this incubation period the conditioned medium was removed by aspiration and replaced with a labelling
15 solution from a commercial cell proliferation kit (XTT, Cell Proliferation kit II, Cat. No. 1 465 015, obtained from Boehringer Mannheim). Once this solution was added an initial absorbance reading was obtained at 450nm, after which the microtitre plate was incubated at 37°C, 5% CO₂ and the absorbance monitored over 4 hours. The proliferative effect of each prototype was evaluated by
20 comparing the absorbance readings measured against the positive and negative controls.

As a positive control, a freeze dried sponge of 45%ORC/55%collagen without any silver was tested, since this was already known to promote fibroblast proliferation.
25

ACTICOAT was used as a negative control, since it was generally known that the effect of silver on cells in culture was toxic with cell death occurring at high concentrations.

30 The results shown in Figure 4 were very surprising. It can be seen that the samples containing 0.2% and 1% of the silver-ORC complex strongly promote fibroblast proliferation. This effect was at least double that observed with collagen/ORC, alone which has been shown previously to stimulate cell

proliferation. This stimulatory effect was apparently limited to lower concentrations of silver-ORC, as concentrations of 2% and above were detrimental to cell growth. The ACTICOAT control in this experiment shows the expected negative effect on fibroblast proliferation.

5

Referring to Figure 6, it can be seen that the sample of collagen/ORC material made with 45%silver-collagen, 10% unmodified collagen, and 45% ORC (Example 9) was also stimulatory for fibroblast proliferation, but not as stimulatory as the collagen/ORC positive control.

10

The Examples have been described for the purpose of illustration only. Many other compositions and methods falling within the scope of the present invention will be apparent to the skilled reader.

CLAIMS

1. A complex of an anionic polysaccharide with silver.
- 5 2. A complex according to claim 1, wherein the complex comprises a salt formed between the anionic polysaccharide and Ag^+ .
3. A complex according to claim 1 or 2, wherein the anionic polysaccharide is a polycarboxylate.
- 10 4. A complex according to any preceding claim, wherein the anionic polysaccharide is selected from the group consisting of alginates, hyaluronates, pectins, carrageenans, xanthan gums, sulfated dextrans, cellulose derivatives, oxidized celluloses and mixtures thereof.
- 15 5. A complex according to any preceding claim, wherein the amount of silver in the complex is from about 1 to about 50% by weight based on the weight of the anionic polysaccharide.
- 20 6. A wound dressing comprising a complex of an anionic polysaccharide with silver.
7. A wound dressing according to claim 6, wherein the wound dressing comprises a freeze-dried sponge sheet, or a woven or nonwoven fibrous sheet, or
25 a gel sheet.
8. A wound dressing according to claim 6 or 7, wherein the wound dressing comprises from about 0.1wt% to about 10wt% of the complex.
- 30 9. A wound dressing according to claim 6, 7 OR 8, wherein the amount of silver in the wound dressing is from about 0.01wt% to about 2wt.%.

FIG. 1

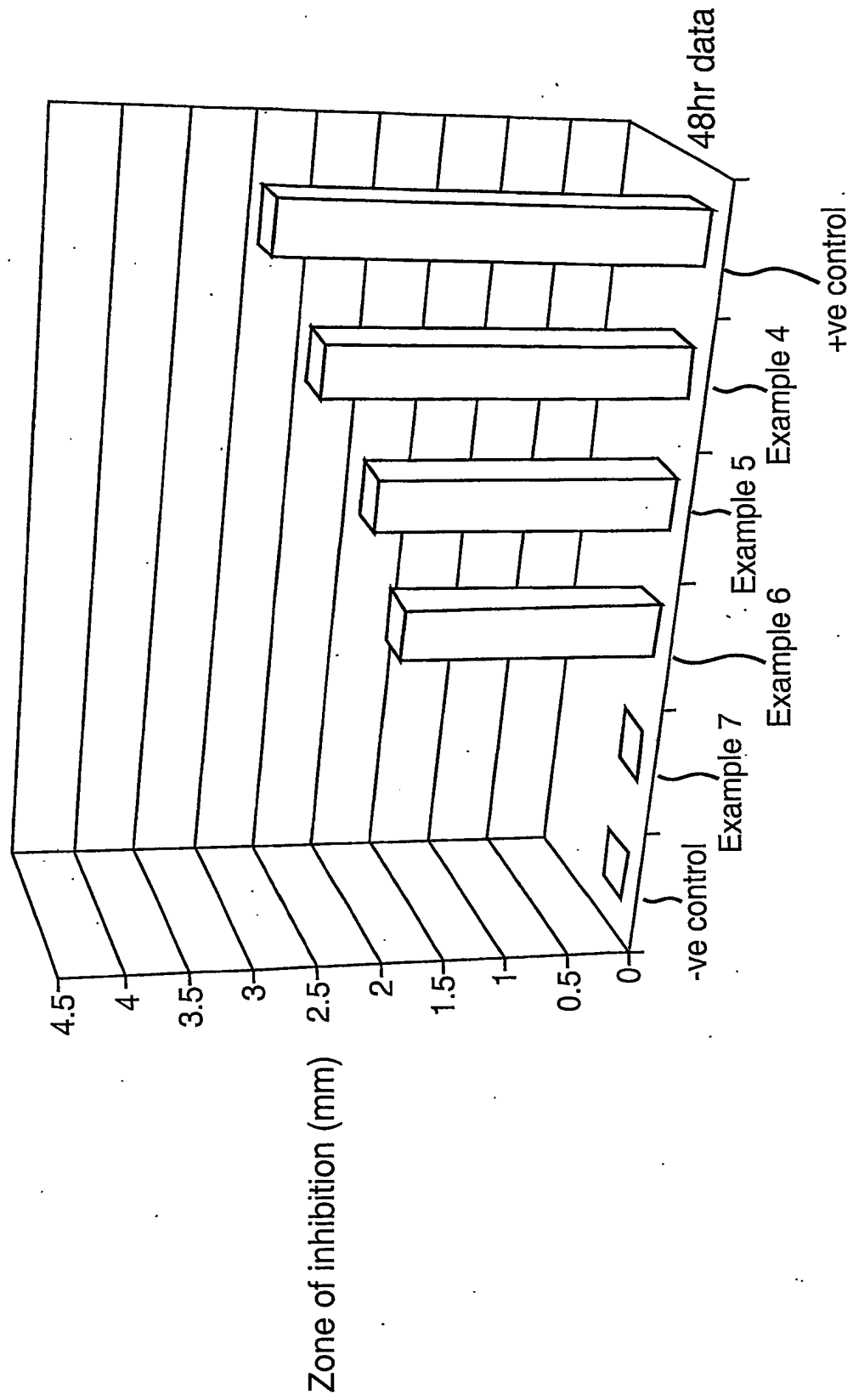


FIG. 2

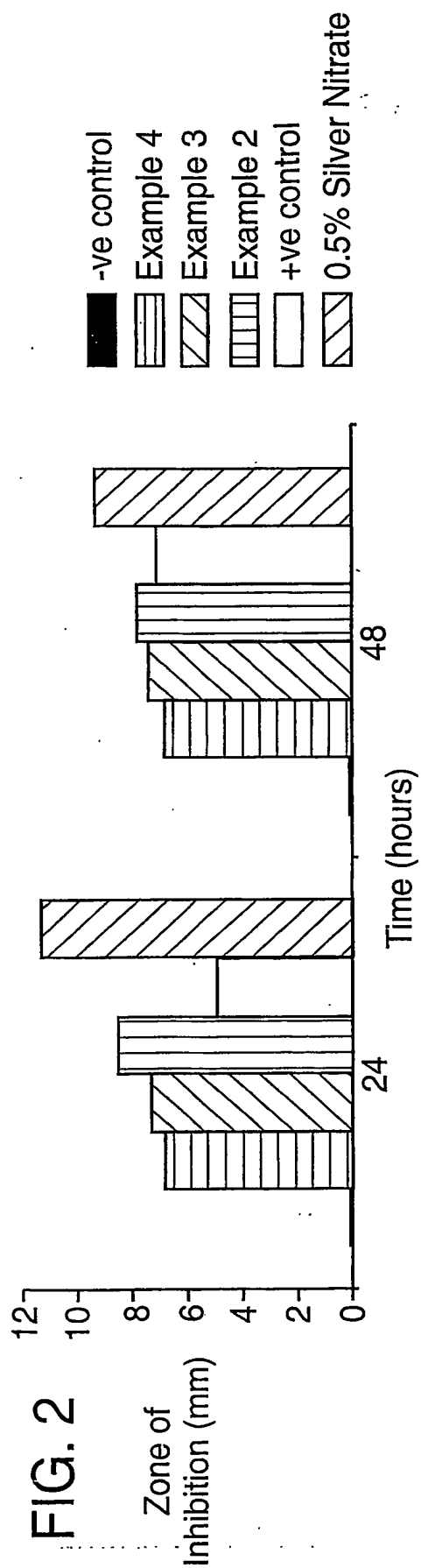


FIG. 3

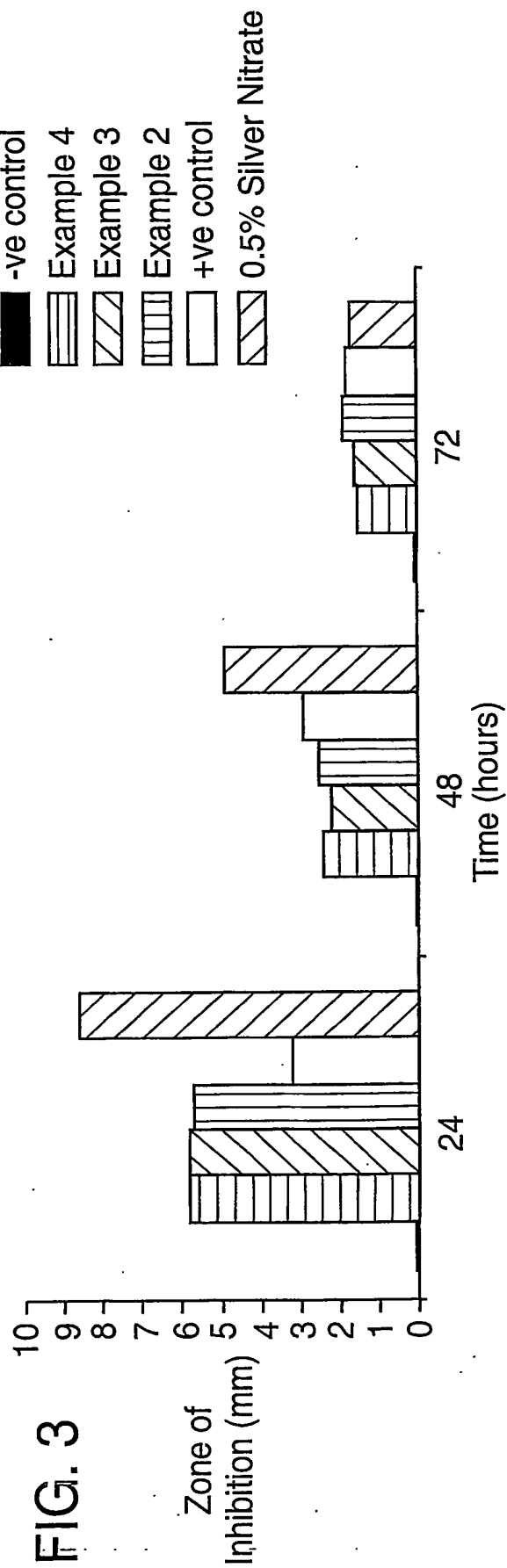


FIG. 4

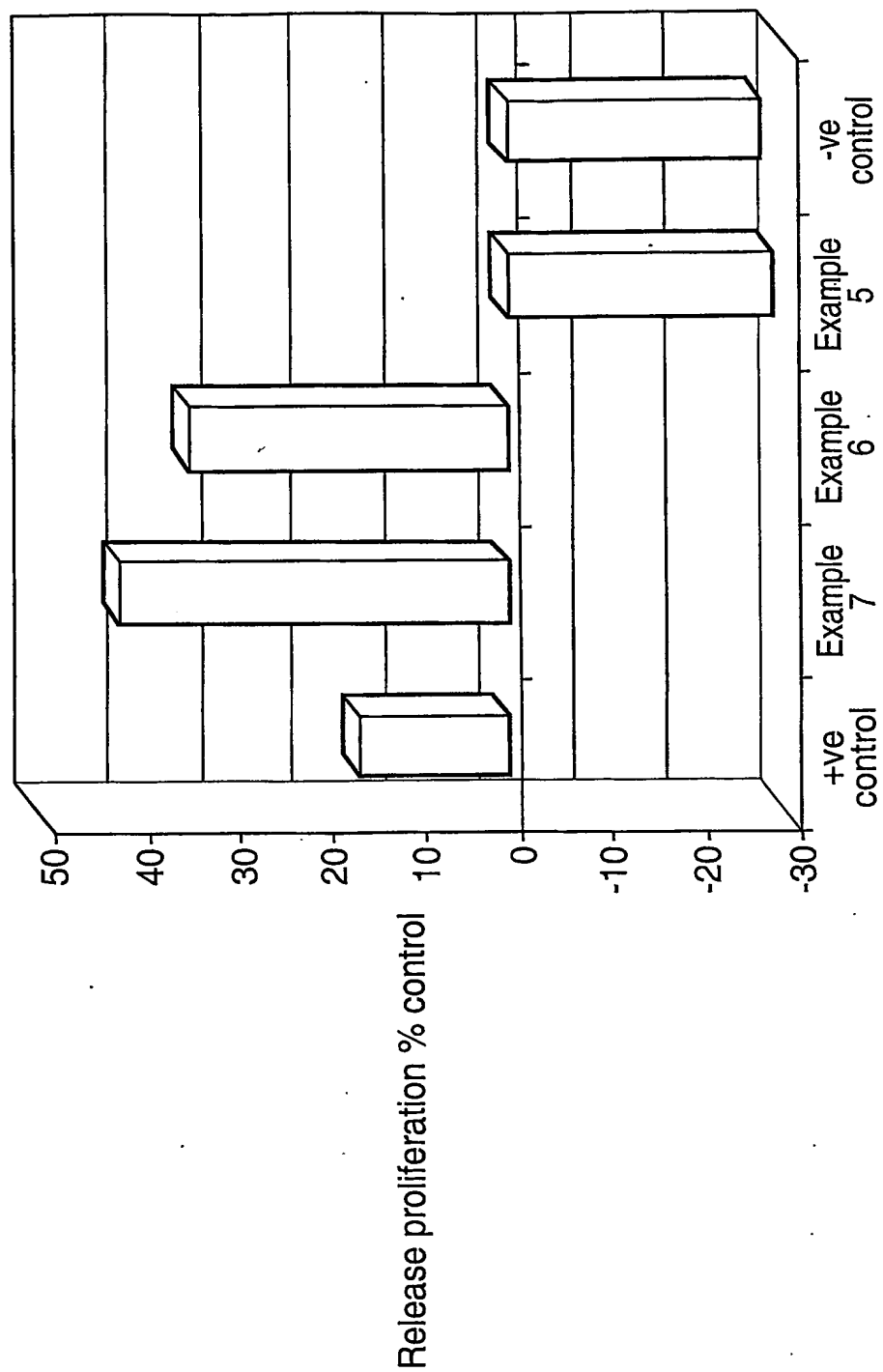


FIG. 5

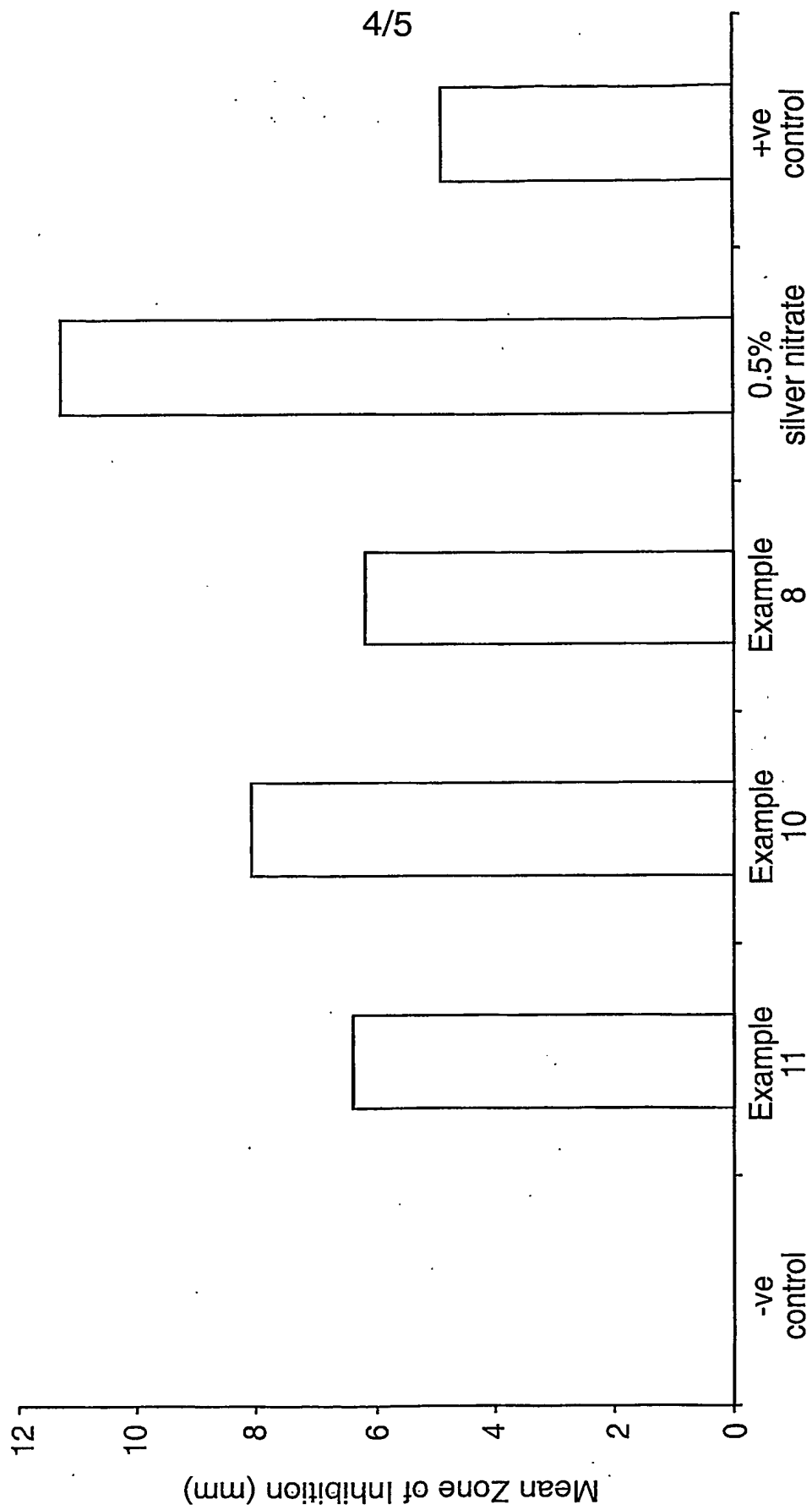
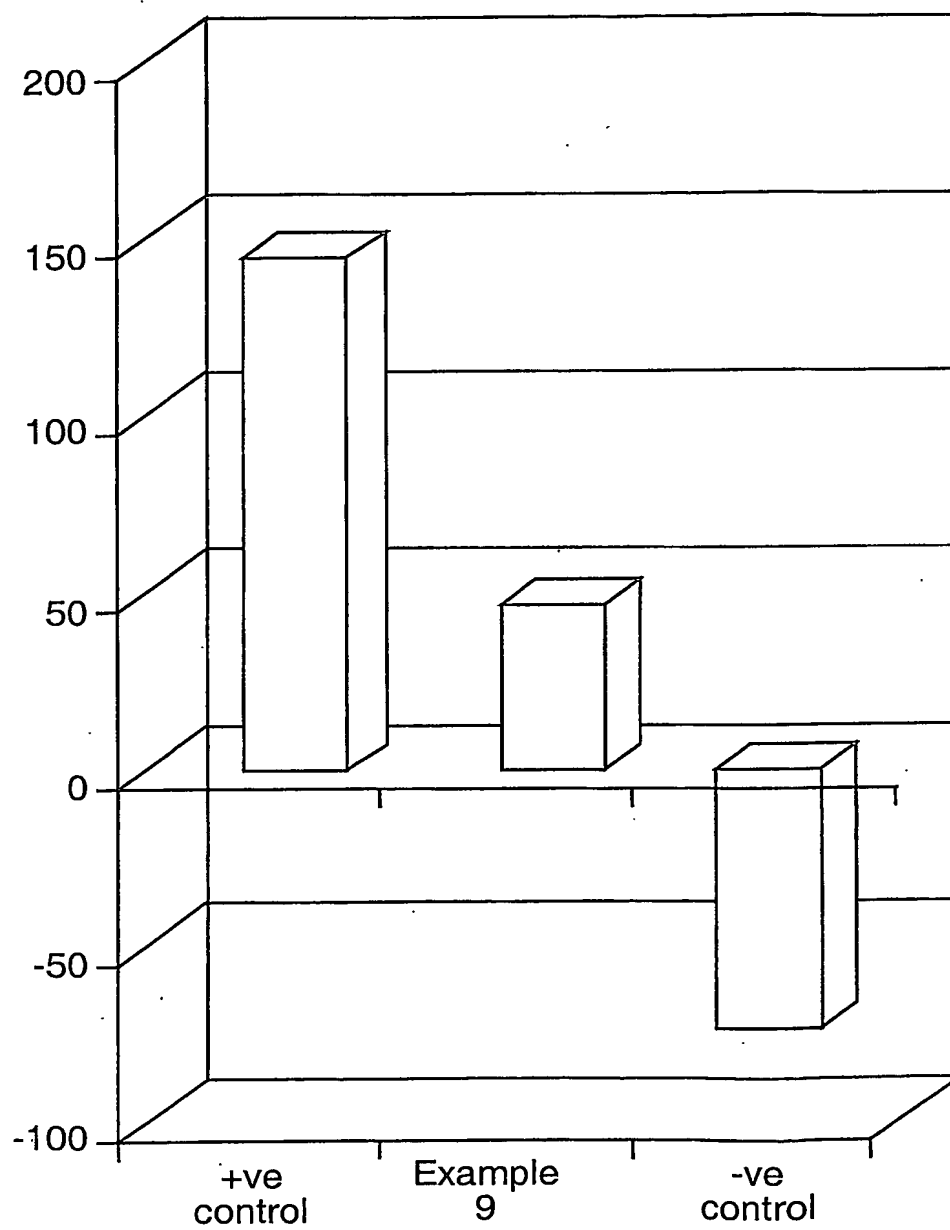


FIG. 6



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